Helicobacter Pylori Stool Antigen ELISA

For the qualitative determination of H. pylori antigen in human stool.

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 30-HPYHU-E01, E10

Size: 96 Wells, 10 x 96 wells

Version: 2024-04-24- ALPCO 4.4

INTENDED USE

The Helicobacter Pylori Stool Antigen ELISA is intended for the qualitative determination of *Helicobacter pylori* in human stool samples. For research use only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

This ELISA is intended for the qualitative determination of *Helicobacter pylori (H. pylori)* antigen in stool. A microtiter plate is coated with antibodies specific for *H. pylori*. The *H. pylori* antigen in the sample is bound by the immobilized antibodies during the first incubation step. At the same time, a peroxidase-labeled antibody binds the *H. pylori* antigen. After a washing step, tetramethylbenzidine is used as a substrate for the peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the color is proportional to the amount of analyte present (sample or control). The results are evaluated by comparison with a cut-off value.

MATERIALS SUPPLIED

Product: 30-HPYHU-E01

Component	Quantity	Preparation
H. pylori antigen Microplate (96 wells)	12 x 8-well strips	Ready to use
Controls Negative and Positive	1 mL each	Ready to use
Wash Buffer Concentrate	2 x 100 mL	10X
Sample Dilution Buffer	100 mL	Ready to use
Conjugate	8 mL	Ready to use
TMB Substrate	15 mL	Ready to use
Stop Solution	15 mL	Ready to use

Product: 30-HPYHU-E10

Component	Quantity	Preparation
H. pylori antigen Microplate (96 wells)	10 Plates: each 12 x 8-well strips	Ready to use
Controls Negative and Positive	10 x 1 mL each	Ready to use
Wash Buffer Concentrate	20 x 100 mL	10X
Sample Dilution Buffer	10 x 100 mL	Ready to use
Conjugate	10 x 8 mL	Ready to use
TMB Substrate	10 x 15 mL	Ready to use
Stop Solution	10 x 15 mL	Ready to use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes for dispensing 10 - 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate reader
- Vortex for sample preparation
- Foil to cover the microplate
- Centrifuge capable of 3000 x g
- Timer

PRECAUTIONS

1. Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are hazardous to health and the environment. Substrates for the enzymatic color reactions may also cause skin and / or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet available from ALPCO upon request.
2. The 10x Wash Buffer concentrate contains surfactants which may cause severe eye irritation in case of eye contact.
   Warning: Causes serious eye irritation
   If in Eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice/attention.
3. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

STORAGE CONDITIONS

The kit should be stored at 2-8°C. The unopened kit is stable until the expiration date on the box label.

SAMPLE HANDLING AND STABILITY

Stool samples are appropriate for use in this assay. The test can be performed on either fresh or frozen stool samples. Raw stool is stable for 7 days at room temperature, 7 days at 2-8 °C, and 7 months at ≤ -20 °C. Avoid more than 3 freeze-thaw cycles.

Extraction of Stool Samples

Stool samples can be extracted by using the following methods:

a) Use a balance to weigh out 100 mg (100 µL for liquid) of stool and add to 500 µL of sample dilution buffer. Homogenize thoroughly using a vortex mixer.

b) Alternatively, weigh out 200 mg (200 µL for liquid) and add 1 mL of sample dilution buffer. Homogenize thoroughly using a vortex mixer.

Centrifuge the suspension for 10 min at 3,000 rpm (2500-3500 x g) for sedimentation of non-soluble particles.

For analysis, pipet 50 µL of supernatant of this stool extract into each well.

Stool extract is stable for 7 days at 2-8°C.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used.

Wash Buffer Concentrate (10X) must be diluted with distilled or deionized water 1:10 before use (100 mL WASHBUF + 900 mL distilled/deionized water) and mixed well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved at room temperature or in a water bath at 37°C. The WASHBUF is stable at 2–8 °C until the expiry date stated on the label. 1X working wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8°C for 1 month.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8°C.

QUALITY CONTROL

Control samples should be analyzed with each run. Results generated from the analysis of control samples should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control samples are outside the acceptable limits given in the QC data sheet. The additional use of external controls is recommended if possible.

ASSAY PROCEDURE

All reagents and microplate strips (while sealed in the foil pouch) should be equilibrated to room temperature (15–30°C) prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. It is recommended to perform tests in duplicate.

Take as many strips as needed from the kit. Unused strips should be stored with the desiccant bag in the closed aluminum packaging at 2 – 8°C. Strips are stable until the expiration date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact ALPCO.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Wash the precoated microtiter strips 5 times with 250 µL of 1X working wash buffer before use (*see Reagent Preparation Section)*. After the final wash step, the inverted microtiter plate should be firmly tapped on absorbent paper.
3. Add 50 µL conjugate into each well.
4. Add 50 µL positive control, negative control, and stool extract supernatant into designated wells and mix by pipetting up and down.
5. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C).
6. Discard the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final wash step, the inverted microtiter plate should be firmly tapped on absorbent paper.
7. Add 100 µL of TMB substrate to each well.

8. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark.*
9. Add 100 µL of stop solution into each well and mix thoroughly.
10. Determine absorption immediately with an ELISA reader at 450nm against 620nm (or 690nm) as a reference. If no reference wavelength is available, read only at 450nm.

* The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

CALCULATION OF RESULTS

Cut-off value = 0.150 OD

The results are categorized according to their absorbances using the cut off value ±0.020 OD as criterion:

OD: 0.130 - 0.170 (cut off value ± 0.020 OD) borderline
OD: >0.170 (> cut off value + 0.020 OD) positive
OD: <0.130 (< cut off value - 0.020 OD) negative

Limitations
Samples with high coefficients of variation of the replicates should be measured again.

PERFORMANCE CHARACTERISTICS

Accuracy- Precision
Repeatability (Intra-Assay)
The repeatability was assessed with 2 stool samples under constant parameters (same operator, measurement system, day, and kit lot).

	Sample 1	Sample 2
Mean (OD)	0.708	0.172
CV (%)	5.1	4.0
n	22	22

Reproducibility (Inter-Assay)
The reproducibility was assessed with 3 stool samples under varying parameters (different operators, measurement systems, days, and kit lots).

	Sample 1	Sample 2	Sample 3
Mean (OD)	0.519	0.354	0.265
CV (%)	7.0	7.1	7.3
n	72	72	72

TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is not recommended to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.

- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. ALPCO cannot be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product.

SHORT ASSAY PROTOCOL

Incubation Time = 1 hour 20 minutes

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running controls and up to 46 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos Ctrl	Pos Ctrl	7	7	15	15	23	23	31	31	39	39
B	Neg Ctrl	Neg Ctrl	8	8	16	16	24	24	32	32	40	40
C	1	1	9	9	17	17	25	25	33	33	41	41
D	2	2	10	10	18	18	26	26	34	34	42	42
E	3	3	11	11	19	19	27	27	35	35	43	43
F	4	4	12	12	20	20	28	28	36	36	44	44
G	5	5	13	13	21	21	29	29	37	37	45	45
H	6	6	14	14	22	22	30	30	38	38	46	46

Ctrl = Control
Numbered wells = Sample